Characterization of Histamine-Releasing Activity: Role of Cytokines and IgE Heterogeneity

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Histamine-releasing factors (HRFs) are a group of cytokines that cause histamine release (HR) from basophils and mast cells. The concept of the priming effect of cytokines and the heterogeneity of IgE involved in the HRF-induced HR have been emphasized in recent years. In this study, we performed a series of experiments to elucidate the above-mentioned hypotheses. The stock HRF were obtained by stimulating mononuclear cells (MNC) with phytohemagglutinin (PHA). Maximal activity was observed 36 hr after culture. By gel filtration, HRF was eluted with a peak activity ranging from 12 to 18 KD. A large portion (75%) of HRF activity could be neutralized by a combination of antibodies against interleukin 1 (IL-1), IL-3, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor-alpha (TNF-α). The stimulation of basophils with 100 ng/ml each of IL-3, IL-6, IL-7, GM-CSF, or TNF-α alone caused 10% HR; however, when the cells were pretreated with 10 ng/ml of either IL-3, IL-6, IL-7, IL-8, TNF-α, or GM-CSF and then stimulated with anti-IgE, a marked increase in HR was regularly observed. The combination of 100 ng/ml each of IL-1, IL-3, IL-8, GM-CSF, and TNF-α could induce only about 20% HR; furthermore, such combinations did not have an additive or synergistic priming effect on anti-IgE-induced HR compared to the effect of single cytokines. Stripping of surface-bound IgE with lactic acid markedly reduced the capacity of basophils to release histamine in response to MNC-HRF and anti-IgE. Passive sensitization of IgE-stripped basophils with high-HRF responders’ serum could restore their responsiveness to both MNC-HRF and anti-IgE, but passive sensitization with low-HRF responders’ serum could restore responsiveness to anti-IgE only. Moreover, passage of MNC-HRF through high-, but not low-HRF, responders’ IgE-Sepharose columns significantly reduced the HR activity of MNC-HRF. Finally, although the eluant could induce only 10% HR, the majority of its HR activity could be restored by the addition of effluent but not by the mixture of IL-1, IL-3, IL-8, GM-CSF, and TNF-α, suggesting the presence of a complex interaction among those cytokines. In summary, MNC-HRF contained at least two types of HRF activity; one was IgE dependent and the other was IgE independent. Cytokines such as IL-1, IL-3, IL-8, GM-CSF, and TNF-α showed a priming effect on histamine release induced by MNC-HRF, although another unidentified cytokine(s) may be also involved.

KEY WORDS: Histamine releasing activity; cytokines; IgE heterogeneity.

INTRODUCTION

Lymphocytes play a very important role in the immediate-type allergic reaction in terms of regulation of IgE production of B lymphocytes and induction of maturation and differentiation of basophil/mast cells (1–3). Dvorak et al. (4) were the first to postulate that lymphocytes might directly activate mast cells and basophils in the delayed-type hypersensitivity reaction. Subsequently, it has been shown that human mononuclear cells (5–8), pulmonary macrophages (9), neutrophils (10), platelets (11), and nasal washings (12) produce a histamine-releasing factor (HRF) that activates mast cells and basophils to release histamine and leukotrienes. Recently, it became apparent that interleukin 1 (IL-1) (13), IL-3 (14–16), IL-5 (17, 18), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (15, 17) could prime, and IL-1 (19), IL-3 (20, 21), IL-8 (22), and GM-CSF (21) could directly activate basophils to release histamine. In addition, the fact that cytokines could play a proinflammatory role by potentiating the effects of various...
secretagogues, e.g., C3a, C5a, anti-IgE, PAF, A23187, NAP-1, and FMLP, further supports the concept of the priming effect of cytokines on the releasability of inflammatory effector cells (23).

More recently, several studies have demonstrated that human basophil responsiveness to HRF generated by alveolar macrophages (24), nasal washings (12, 25), mononuclear cells (MNCs) (25), T cells (25), B cells (25), and bronchoalveolar lavage (25) was dependent upon IgE antibody which could be obtained only from a selected severe group of allergic patients, and these results reflected the heterogeneity of the IgE molecule, i.e., unique species of IgE responded to HRF (IgE\(^+\)), while others did not. Furthermore, nonresponder basophils could be made to become responder by being stripped of surface IgE, followed by passive sensitization with responder IgE. This phenomenon suggested that HRF could directly interact with IgE\(^+\) (24, 25).

Kuna et al. (26) and we (27) recently reported that successful immunotherapy could suppress the production of and responsiveness to HRF in asthmatic children. This study was conducted (i) to characterize the physicochemical property of MNC-HRF, (ii) to explore the relationship between HRF and a variety of cytokines, and (iii) to test the hypothesis of IgE heterogeneity.

**MATERIALS AND METHODS**

**Preparation of Peripheral Blood Mononuclear Cells (MNCs)**

Peripheral blood mononuclear cells were prepared by the standard Ficoll/Hypaque density-gradient centrifugation of Boyum (28). MNCs were washed with Hanks' balanced salt solution (HBSS; GIBCO) three times. The contaminating platelets were removed by centrifugation for 7 min at 150g. The MNCs were finally resuspended at a concentration of \(5 \times 10^6\) cells/ml in complete culture medium (RPMI 1640 containing 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 \(\mu\)g/ml streptomycin, and 2 \(mM\) l-glutamine; GIBCO). The platelet concentration in the final cell suspension was usually threefold to fivefold of the concentration of MNCs.

**Preparation of Stock HRF**

Human peripheral blood MNCs from 10 healthy individuals were pooled, stimulated with different concentrations of phytohemagglutinin (PHA) (Wellcome), for varying period of time in a 37°C, 5% CO\(_2\), humidified incubator. The supernatant was collected by centrifugation at 1000g for 20 min, and the sterile cell-free supernatants were ultrafiltered by using an Amicon YM 100 membrane and then concentrated 50-fold with an Amicon YM 5 membrane. The PHA contained in the supernatant was removed by incubation with 0.05 \(M\) \(\alpha\)-methyl mannoside (Sigma). For purification and characterization of HRF, a large batch was prepared from the leukocyte concentrates of 200 normal donors. The MNCs were separated, stimulated with 2 \(\mu\)g/ml of PHA for 4 hr, washed three times with HBSS, and then cultured for an additional 32 hr. Cell-free supernatants were pooled and concentrated as described above.

**Lymphoproliferation Assay**

Lymphoproliferation was done by incubating \(1 \times 10^6\) MNCs/well in triplicate in round-bottom 96-well microtiter plates (Costar) and in the presence of 2 \(\mu\)g/ml PHA. Two microcuries of tritiated thymidine (NEN) was added during the final 18 hr of 3-day culture. The cells were harvested using a semiautomatic cell harvester. Radioactivity was determined on a \(\beta\) counter.

**Sephadex G-75 Chromatography**

Two milliliters of concentrated HRF was applied to a \(5 \times 100\)-cm column of Sephadex G-75 superfine equilibrated with 0.01 \(M\) phosphate buffer containing 0.15 \(M\) NaCl. The column was eluted at a flow rate of 12 ml/hr. Two-milliliters fractions were collected and absorbance at 280 nm was checked. The fractions were then assessed for their capacity to induce histamine release from the leukocytes of a high-HRF responder. The fractions showing the highest HR activity were pooled, aliquoted, frozen at \(-70^\circ\)C, and used as partially purified HRF.

**Preparation of Basophils**

Leukocytes were separated from venous blood by dextran sedimentation. In some experiments, purified basophils were prepared according to the method of Hirai et al. (18). Briefly, whole leukocytes were fractionated by discontinuous Percoll (Pharmacia) gradients with densities of 1.072, 1.082, and 1.100 g/ml. The interface layer, at a density of \(\leq 1.082\), contained most of the basophilic leuko-